Machine-Learning-Based Analysis in Genome-Edited Cells Reveals the Efficiency of Clathrin-Mediated Endocytosis.

Public Summary:

A fundamental property of cell biology is the ability to internalize lipids, signaling molecules such as growth factors and nutrients ("cargo") through clathrin-mediated endocytosis (CME). During CME, cellular proteins and cargo molecules assemble at a region of the plasma membrane and form a coated pit which is subsequently pinched off to deliver the cargo molecules to the inside of the cell. Previous studies have reported that this process is highly inefficient, with a large fraction of potential CME sites failing to form vesicles, Here, we developed a robust tool to distinguish authentic CME sites from false CME sites. . When we excluded the false CME sites from our analyses, we found that the vast majority (~90%) of the authentic CME sites form vesicles, in contrast to previous conclusions.

Scientific Abstract:

Cells internalize various molecules through clathrin-mediated endocytosis (CME). Previous live-cell imaging studies suggested that CME is inefficient, with about half of the events terminated. These CME efficiency estimates may have been confounded by overexpression of fluorescently tagged proteins and inability to filter out false CME sites. Here, we employed genome editing and machine learning to identify and analyze authentic CME sites. We examined CME dynamics in cells that express fluorescent fusions of two defining CME proteins, AP2 and clathrin. Support vector machine classifiers were built to identify and analyze authentic CME sites. From inception until disappearance, authentic CME sites contain both AP2 and clathrin, have the same degree of limited mobility, continue to accumulate AP2 and clathrin over lifetimes > approximately 20 s, and almost always form vesicles as assessed by dynamin2 recruitment. Sites that contain only clathrin or AP2 show distinct dynamics, suggesting they are not part of the CME pathway.